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Potential Male and Female Reproductive Toxicants: Applying the Key Characteristics Approach

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With more than 40,000 chemicals currently in use in the United States,[1] it would be a seemingly impossible task to determine how all of them affect human health. With so much uncertainty, how should investigators prioritize which chemicals to study? A new "key characteristics" approach may help. Two papers recently published in *Environmental Health Perspectives* outlined this approach in identifying key characteristics of chemicals that cause reproductive toxicity in females[2] and males.[3]

Identifying key characteristics enables toxicologists to flag chemicals that have qualities associated with specific forms of toxicity. In 2012, an international working group organized by the International Agency for Research on Cancer compiled the first list of key characteristics when they identified 10 properties shared by carcinogenic chemicals.[4] Practical and objective, the key characteristics of carcinogens have been invoked in recent Proposition 65 evaluations by the California Environmental Protection Agency (EPA).[5,6,7] The state conducts these evaluations to determine whether agents should be added to the Proposition 65 list of chemicals known to cause cancer, birth defects, or other reproductive harm.

To identify the key characteristics of male and female reproductive toxicants, a working group of experts convened at the University of California, Berkeley, in March 2018. The meeting organized by Lauren Zeise, director of the California EPA's Office of Environmental Health Hazard Assessment, and Martyn Smith, director of Berkeley's Superfund Research Program Center. The members of the group brought expertise in mechanistic toxicology, obstetrics and gynecology, occupational and environmental medicine, urology, epidemiology, risk assessment, and regulation of hazardous chemicals, among other areas.

"The meeting started out . . . with presentations by several of the participants," says Ulrike Luderer, director of the Center for Occupational and Environmental Health at the University of California, Irvine, School of Medicine, and coauthor of the paper focused on female reproduction.[2] The initial presentations proposed possible key characteristics of male and female reproductive toxicants and endocrine disruptors, which were discussed and debated by the convened experts. "We then split up into three groups based on people's expertise to discuss the proposed key characteristics, begin refining them, and identifying example chemicals," Luderer says.

The workshop was followed by a series of conference calls to finalize details. The male toxicant group settled on 8 key characteristics,[3] while the female group identified 10,[2] narrowing it down from an earlier 12.

"In the opening paragraphs of these two articles . . . we sort of lay the groundwork of how fertility and other forms of reproductive health are being injured by environmental chemicals and that

Two expert groups developed separate lists of key characteristics for male and female reproductive toxicants because of the substantial differences in the sexes' reproductive systems. But the two lists share many similarities, such as the ability to cause genotoxicity, alter hormone production and/or hormone receptor function, induce epigenetic changes, and cause oxidative stress. Image: © iStockphoto/levkr.

the evidence for this is increasing," Smith said in a recent *EHP* podcast.[8] Smith was a coauthor on both of the new reproductive toxicity papers[2,3] as well as the earlier carcinogen report.[4]

Smith says that, while some people recommend focusing on broad classes of chemicals, such an approach is unsatisfactory to most chemists. "[T]hey know that just changing the structure very slightly will change the properties of the chemical," he noted in the podcast. "We're just hoping that the key characteristics concept can help us with this by looking at thousands of chemicals in a particular, uniform, standardized way."[5]

Initially, the experts considered tackling male and female reproductive characteristics jointly but opted to separate the groups due to the substantial differences in the two sexes' reproductive systems. In the end, the selected traits overlapped, although differences did emerge.

For female reproductive tissues, a likely reproductive toxicant may 1) alter hormone receptor signaling and/or alter reproductive hormone production, secretion, or metabolism; 2) be genotoxic; 3) induce genetic alterations; 4) cause mitochondrial dysfunction; 5) induce oxidative stress; 6) alter immune function; 7) alter cell signal transduction; 8) alter direct cell–cell interactions; 9) alter survival, proliferation, cell death, or metabolic pathways; and/or 10) alter microtubules and associated structures.

For male reproductive tissues, a likely reproductive toxicant may 1) alter germ cell development, function, or death; 2) alter somatic cell development, function, or death; 3) alter production and levels of reproductive hormones; 4) alter hormone receptor levels/functions; 5) be genotoxic; 6) induce epigenetic alterations; 7) induce oxidative stress; and/or 8) induce inflammation.

"The KC [key characteristics] approach is a way to start organizing evidence so that it can be more readily evaluated by experts in the field to judge whether the evidence is strong and clear [for reproductive toxicity]," says Gail Prins, a urology professor at the University of Illinois at Chicago and coauthor of the male toxicant paper.[3] "It is *not* a checklist where, for example, you hit three to four KCs and then it's a reproductive toxicant. If there is a specific chemical that only checks off one KC, but the evidence from human and animal studies is clear that it is adversely affecting male [or female] reproductive health, then that could be sufficient."

Another strength of the approach, Prins says, is that investigators do not need to know the mechanism of action. "For example, if it has been determined that a chemical causes more sperm cells to die, one does not need to know first what triggered that event—what receptor was involved, whether it was direct on the sperm or indirect by first affecting another system that controls sperm formation, et cetera," says Prins. "Knowing that it's killing the sperm is sufficient to add it to KC number one." The approach also allows experts to group evidence together, whether from human epidemiological studies, animal studies, cell-based research, or high-throughput assays.

"This [framework] could prove helpful," says Kembra Howdeshell, a health scientist with the Office of Health Assessment and Translation at the National Institute of Environmental Health Sciences National Toxicology Program. The approach may even reduce the number of animals needed for testing potential toxicity of new chemicals. For example, the key characteristics could be evaluated in tests that use cell culture, tissue culture, or short-term *in vivo* assays. However, Howdeshell says, the applicability of this approach to a testing program would only be that of a first tier to identify potential reproductive toxicants that would require more in-depth testing.

She adds, "While some of the key characteristics are well characterized and their mechanisms of action are known, other characteristics are less studied and would benefit from additional research to better understand their downstream effects on reproductive outcomes in animal models and their applicability to human health."

In 2017, the National Academies of Sciences, Engineering, and Medicine recommended that the key characteristics approach be used to identify traits of chemicals leading to other end points.[9] The conclusions of a third group at the March 2018 meeting, which focused on endocrine disruption, were published in *Nature Reviews Endocrinology* in November 2019.[10] Experts also met in September 2019 to identify key characteristics for neurotoxicity and developmental neurotoxicity, while meetings to discuss cardiotoxicants and toxic bioactive chemicals are planned for later in 2020.

Wendee Nicole has written for *Discover*, *Scientific American*, and other publications.

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